

Detection of UCP1 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)
[3% Hydrogen Peroxide](#)
[1% BSA Diluent](#)
[1X Trilogy](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Blocking Serum: Normal Donkey Serum
Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 017-000-011

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Rabbit Polyclonal to UCP1 Antibody
Abcam, Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab23841

Negative Serum Control: Normal Rabbit Serum
Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Secondary Antibody: Donkey Anti-Rabbit IgG (H+L) Biotin-SP-Conjugated
Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 711-065-152

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Mammary – Brown adipose tissue
Stain Localization: Mitochondrion inner membrane

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Steamer

Add distilled water to the bottom portion of the steamer.

Preheat 200ml of 1X Trilogy solution in a Tissue Tek® container in the steamer between 95°C and 100°C. Immerse a full rack of slides into the trilogy and place the lid back on the steamer.

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Steam the slides for 20 minutes.

Remove container from steamer and cool for 20 minutes.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse slides in 2 changes of 1X Wash Buffer for 5 minutes each.

6. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:250 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match the protein concentration of the primary antibody. Make a 1:250 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the donkey anti-rabbit secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

Exp. Date _____ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

14. Apply the DAB chromagen. Incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # _____ Exp. Date _____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip